

**Amendments to the Specification:**

Please replace the paragraph at page 14, lines 24-25, with the following amended paragraph:

The binding of the wild-type or mutant Hu1D10-IgG2M3 antibodies to Raji cells was analyzed in a FACS™ binding assay, as described in Example 8.

Please replace the paragraph at page 14, lines 28-29, with the following amended paragraph:

The binding of the wild-type or mutant Hu1D10-IgG1 antibodies to Raji cells was analyzed in a FACS™ binding assay, as described in Example 8.

Please replace the paragraph beginning at page 26, line 6, with the following amended paragraph:

In particular embodiments, the modified antibodies have in vivo therapeutic and/or prophylactic uses. Examples of therapeutic and prophylactic prophylactic antibodies which may be so modified include, but are not limited to, SYNAGIS® (Medimmune, Md.) which is a humanized anti-respiratory syncytial virus (RSV) monoclonal antibody for the treatment of patients with RSV infection; HERCEPTIN® (Trastuzumab) (Genentech, Calif.) which is a humanized anti-HER2 monoclonal antibody for the treatment of patients with metastatic breast cancer; REMICADE® (infliximab) (Centocor, Pa.) which is a chimeric anti-TNF- $\alpha$  monoclonal antibody for the treatment of patients with Crohn's disease; REOPRO® (abciximab) (Centocor) which is an anti-glycoprotein IIb/IIIa receptor on the platelets for the prevention of clot formation; ZENAPAX® (daclizumab) (Roche Pharmaceuticals, Switzerland) which is an immunosuppressive, humanized anti-CD25 monoclonal antibody for the prevention of acute renal allograft rejection. Other examples are a humanized anti-CD18 F(ab')2 (Genentech); CDP860 which is a humanized anti-CD18 F (ab')2 (Celltech, UK); PR0542 which is an anti-HIV

gp120 antibody fused with CD4 (Progenics/Genzyme Transgenics); OSTAVIR™ which is a human anti Hepatitis B virus antibody (Protein Design Labs/Novartis); PROTOVIR™ which is a humanized anti-CMV IgG1 antibody (Protein Design Labs/Novartis); IC14 which is an anti-CD14 antibody (ICOS); AVASTIN™ which is a humanized anti-VEGF IgG1 antibody (Genentech); ERBITUX™ which is a chimeric anti-EGFR IgG antibody (ImClone Systems); VITAXIN™ which is a humanized anti- $\alpha$ V $\beta$ 3 integrin antibody (Applied Molecular Evolution/Medimmune); Campath-1H/LDP-03 which is a humanized anti CD52 IgG1 antibody (Leukosite); ZAMYL™ which is a humanized anti-CD33 IgG antibody (Protein Design Labs/Kanebo); RITUXAN™ which is a chimeric anti-CD20 IgG1 antibody (IDEC Pharmaceuticals/Genentech, Roche/Zenyaku); LYMPHOCIDE™ which is a humanized anti-CD22 IgG antibody (Immunomedics); REMITOGEN™ which is a humanized anti-HLA-DR antibody (Protein Design Labs); ABX-IL8 which is a human anti-IL8 antibody (Abgenix); RAPTIVA™ which is a humanized IgG1 antibody (Genetech/Xoma); ICM3 which is a humanized anti-ICAM3 antibody (ICOS); IDEC-114 which is a primatized anti-CD80 antibody (IDEC Pharmaceuticals/Mitsubishi); IDEC-131 which is a humanized anti-CD40L antibody (IDEC/Eisai); IDEC-151 which is a primatized anti-CD4 antibody (IDEC); IDEC-152 which is a primatized anti-CD23 antibody (IDEC/Seikagaku); NUVION™ which is a humanized anti-CD3 IgG (Protein Design Labs); 5G1.1 which is a humanized anti-complement factor 5 (C5) antibody (Alexion Pharmaceuticals); HUMIRA™ which is a human anti-TNF- $\alpha$  antibody (CAT/BASF); CDP870 which is a humanized anti-TNF- $\alpha$  Fab fragment (Celltech); IDEC-151 which is a primatized anti-CD4 IgG1 antibody (IDEC Pharmaceuticals/Smith-Kline Beecham); MDX-CD4 which is a human anti-CD4 IgG antibody (Medarex/Eisai/Genmab); CDP571 which is a humanized anti-TNF- $\alpha$  IgG4 antibody (Celltech); LDP-02 which is a humanized anti- $\alpha$ 4 $\beta$ 7 antibody (LeukoSite/Genentech); OrthoClone OKT4A which is a humanized anti-CD4 IgG antibody (Ortho Biotech); ANTOVA™ which is a humanized anti-CD40L IgG antibody (Biogen); ANTEGRENTM which is a humanized anti-VLA-4 IgG antibody (Elan); MDX-33 which is a human anti-CD64 (Fc $\gamma$ R) antibody (Medarex/Centeon); SC<sub>H</sub>55700 which is a humanized anti-IL-5 IgG4 antibody (Celltech/Schering); SB-240563 and SB-240683 which are

humanized anti-IL-5 and IL-4 antibodies, respectively (SmithKline Beecham); rhuMab-E25 which is a humanized anti-IgE IgG1 antibody (Genentech/Novartis/Tanox Biosystems); IDEC-152 which is a primatized anti-CD23 antibody (IDEC Pharmaceuticals); SIMULECT™ which is a chimeric anti-CD25 IgG1 antibody (Novartis Pharmaceuticals); LDP-01 which is a humanized anti- $\beta$ 2-integrin IgG antibody (Leukosite); CAT-152 which is a human anti-TGF- $\beta$ 2 antibody (Cambridge Antibody Technology); and Corsevin M which is a chimeric anti-Factor VII antibody (Centocor).

Please replace the paragraph beginning at page 50, line 7, with the following amended paragraph:

To generate the T250I T250I and T250L mutants, the mutagenesis primers KH4 (5'-GAC CTC AGG GGT CCG GGA GAT CAT GAG AAK GTC CTT GG-3') (SEQ ID NO: 81) and KH3 (5'-CTC ATG ATC TCC CGG ACC CCT GAG GTC-3') (SEQ ID NO: 82) were used, where K=G or T. To generate the T250C and T250G mutants, the mutagenesis primers KH5 (5'-GAC CTC AGG GGT CCG GGA GAT CAT GAG GCM GTC CTT GG-3') (SEQ ID NO: 83) and KH3 were used, where M=A or C. To generate the T250N and T250Q mutants, the mutagenesis primers KH6 (5'-GAC CTC AGG GGT CCG GGA GAT CAT GAG NTK GTC CTT GG-3') (SEQ ID NO: 84) and KH3 were used, where K=G or T, and N=A, C, G or T. The first round of PCR used outside primer msc g2-1 and KH4, KH5 or KH6 for the left-hand fragment, and outside primer MGD-1 (5'-GCC AGG ATC CGA CCC ACT-3') (SEQ ID NO: 85) and KH3 for the right-hand fragment. The PCR reactions were done using the Expand™ High Fidelity PCR System (Roche Diagnostics Corporation) by incubating at 94° C for 5 minutes, followed by 25 cycles of 94° C for 5 seconds, 60° C for 5 seconds and 72° C for 60 seconds, followed by incubating at 72° C for 7 minutes. The PCR products were run on a low-melting point agarose gel, excised from the gel, and melted at 70° C. The second round of PCR to combine the left-hand and right-hand fragments was done as described above, using outside primers msc g2-1 and MGD-1, by incubating at 94° C for 5 minutes, followed by 35 cycles of

94° C for 5 seconds, 60° C for 5 seconds and 72° C for 105 seconds, followed by incubating at 72° C for 7 minutes. The final PCR products were run on a low-melting point agarose gel and DNA fragments of the expected size were excised and purified using the QIAquick™ Gel Extraction Kit (QIAGEN®). The purified fragments were digested with PinAI and BamHI, gel-purified as described above, and cloned between the corresponding sites in pVAg2M3-OST577.

Please replace the paragraph beginning at page 62, line 19, with the following amended paragraph:

NS0 cells were stably transfected with pDL208. Approximately  $1 \times 10^7$  cells were washed once and resuspended in 1 ml of plain DMEM, transferred to a Gene Pulser™ Cuvette (Bio-Rad® Laboratories), and incubated on ice for 10 minutes. Forty µg of plasmid pDL208 was linearized with FspI and gently mixed with the cells on ice, then the cells were electroporated by pulsing twice using a Gene Pulser™ II (Bio-Rad® Laboratories) set at 1.5 kV, 3 µF, and returned to ice for 10 minutes. The cells were diluted in 20 ml of DMEM, 10% FBS, and plated in two 96-well plates at 100 µl/well. The medium was replaced after 48 hours with MPA selection medium. Mycophenolic acid-resistant NS0 transfectants from wells apparently containing single colonies were expanded in MPA selection medium and screened after about 3 weeks by FACSA FACS™. Approximately  $1.5 \times 10^5$  cells/test were incubated in 100 µl of FACS Staining Buffer (FSB) (PBS, 1% FBS, 0.1% NaN<sub>3</sub>) containing 10 µg/ml of biotinylated mouse anti-human β2-microglobulin antibody (Chromaprobe, Inc., Aptos, Calif.) for 1 hour on ice. The cells were washed once with 4 ml of FSB, then incubated in 25 µl of FSB containing 20 µg/ml of streptavidin-FITC conjugate (Southern Biotechnology Associates, Inc.) for 30 minutes on ice in the dark. The cells were washed once with 4 ml of FSB, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to human β2m using a FACScan™ flow cytometer (BD® Biosciences, San Jose, Calif.). Several clones with the highest apparent staining were subcloned using a FACStar cell sorter (BD® Biosciences), expanded in DMEM, 10% FBS, 2

mM L-glutamine, and retested by FACS™ as described above. One subclone, designated NS0 HuFcRn (memb), clone 7-3, was used in subsequent binding assays.

Please replace the paragraph beginning at page 63, line 16, with the following amended paragraph:

Concentrated OST577-IgG2M3 supernatants were tested in a single-point competitive binding assay for binding to human FcRn on cell line NS0 HuFcRn (memb), clone 7-3. Approximately  $2 \times 10^5$  cells/test were washed once in FACS Binding Buffer (FBB) (PBS containing 0.5% BSA, 0.1% NaN<sub>3</sub>), pH 8.0, once in FBB, pH 6.0, and resuspended in 120 µl of pre-mixed biotinylated OST577-IgG2M3 antibody (8.3 µg/ml) and concentrated supernatant (containing 8.3 µg/ml of competitor antibody) in FBB, pH 6.0. The cells were incubated for 1 hour on ice, washed twice in FBB, pH 6.0, and resuspended in 25 µl of streptavidin-RPE conjugate (BioSource International) diluted to 2.5 µg/ml in FBB, pH 6.0. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB, pH 6.0, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACSCalibur™ flow cytometer (BD® Biosciences). Mean channel fluorescence (MCF) of each mutant was compared to that of the wild-type antibody and plotted using Excel (Microsoft® Corporation, Redmond, Wash.).

Please replace the paragraph beginning at page 63, line 30, with the following amended paragraph:

A dilution series of each purified OST577-IgG2M3 antibody was competed against biotinylated HuEP5C7-IgG2M3 antibody (He et al., J. Immunol. 160:1029-1035 (1998)) for binding to human FcRn on cell line NS0 HuFcRn (memb), clone 7-3. For initial screening experiments, approximately  $2 \times 10^5$  cells/test were washed once in FSB, pH 6.0, and resuspended in 100 µl of pre-mixed biotinylated HuEP5C7-IgG2M3 antibody (10 µg/ml) and OST577-

IgG2M3 competitor antibody (twofold serial dilutions from 208 µg/ml to 0.102 µg/ml) in FSB, pH 6.0. The cells were incubated with the antibody mixture for 1 hour on ice, washed twice in FSB, pH 6.0, and resuspended in 25 µl of streptavidin-RPE conjugate (BioSource International) diluted to 2.5 µg/ml in FSB, pH 6.0. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FSB, pH 6.0, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACScan™ flow cytometer (BD® Biosciences). Mean channel fluorescence (MCF) was plotted against competitor concentration, and IC<sub>50</sub> values were calculated using GraphPad Prism® (GraphPad™ Software, Inc., San Diego, Calif.). For consistency, the IC<sub>50</sub> values shown in the Tables are based on the final competitor concentrations.

Please replace the paragraph beginning at page 64, line 26, with the following amended paragraph:

A dilution series of each purified OST577-IgG2M3 antibody was competed against biotinylated OST577-IgG2M3 antibody for binding to rhesus FcRn on cell line NS0 RhFcRn, clone R-3. In one group of experiments, approximately  $2 \times 10^5$  cells/test were washed once in FBB, pH 8.0, and once in FBB, pH 6.0, then resuspended in 120 µl of pre-mixed biotinylated OST577-IgG2M3 antibody (8.3 µg/ml) and OST577-IgG2M3 competitor antibody (twofold serial dilutions from 208 µg/ml to 0.102 µg/ml) in FBB, pH 6.0. The cells were incubated with the antibody mixture for 1 hour on ice, washed twice in FBB, pH 6.0, and resuspended in 25 µl of streptavidin-RPE conjugate (BioSource International) diluted to 2.5 µg/ml in FBB, pH 6.0. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB, pH 6.0, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACSCalibur™ flow cytometer (BD® Biosciences). Another group of experiments was done in 200 µl of pre-mixed biotinylated OST577-IgG1 antibody (5.0 µg/ml) and OST577-IgG1 competitor antibody (threefold serial dilutions starting from 500 µg/ml) in FBB, pH 6.0, as described above.

Please replace the paragraph beginning at page 70, line 26, with the following amended paragraph:

Purified OST577-IgG2M3 antibodies were tested for binding to human FcRn on cell line NS0 HuFcRn (memb), clone 7-3, or to untransfected NS0 cells in FBB at pH 6.0. Approximately  $2 \times 10^5$  cells/test were washed once in FBB, pH 8.0, and once in FBB, pH 6.0, then resuspended in 100  $\mu$ l of antibody at a concentration of 11  $\mu$ g/ml in FBB, pH 6.0. The cells were incubated with antibody for 1 hour on ice, washed twice in FBB, pH 6.0, and resuspended in 25  $\mu$ l of goat anti-human IgG RPE-conjugated antibody (Southern Biotechnology Associates, Inc.) diluted to 5  $\mu$ g/ml in FBB, pH 6.0. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB, pH 6.0, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACScan™ flow cytometer (BD® Biosciences). Mean channel fluorescence (MCF) of each mutant was plotted using Excel (Microsoft® Corporation).

Please replace the paragraph beginning at page 71, line 6, with the following amended paragraph:

A dilution series of each purified OST577-IgG2M3 antibody was competed against biotinylated OST577-IgG2M3 antibody for binding to human FcRn on cell line NS0 HuFcRn (memb), clone 7-3 at 37° C. Approximately  $2 \times 10^5$  cells/test were washed once in FBB, pH 8.0, and once in FBB, pH 6.0, then resuspended in 100  $\mu$ l of pre-mixed biotinylated OST577-IgG2M3 antibody (10  $\mu$ g/ml) and OST577-IgG2M3 competitor antibody (twofold serial dilutions, from 208  $\mu$ g/ml to 0.102  $\mu$ g/ml) in FBB, pH 6.0. The cells were incubated with the antibody mixture for 1 hour at 37° C., washed twice in FBB, pH 6.0, and resuspended in 25  $\mu$ l of streptavidin-RPE conjugate (BioSource International) diluted to 2.5  $\mu$ g/ml in FBB, pH 6.0. After incubation for 30 minutes in the dark, the cells were washed twice in FBB, pH 6.0, and

resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACScan™ flow cytometer (BD® Biosciences). Mean channel fluorescence (MCF) was plotted against competitor concentration, and IC<sub>50</sub> values were calculated using GraphPad Prism® (GraphPad™ Software).

Please replace the paragraph beginning at page 71, line 21, with the following amended paragraph:

Purified OST577-IgG2M3 and OST577-IgG1 mutant antibodies were compared to the respective wild-type antibodies for binding to human FcRn and then released at various pH values in single-point binding and release assays using cell line NS0 HuFcRn (memb), clone 7-3. Approximately  $2 \times 10^5$  cells/test were washed once in FBB, pH 8.0, and once in FBB, pH 6.0, then resuspended in 100 µl of purified antibody (10 µg/ml) in FBB, pH 6.0. The cells were incubated for 1 hour on ice, washed twice in FBB, pH 6.0, 6.5, 7.0, 7.5, or 8.0, and resuspended in 25 µl of goat F(ab')<sub>2</sub> anti-human IgG FITC-conjugated antibody (Southern Biotechnology Associates, Inc.) diluted to 1.25 µg/ml in FBB of the appropriate pH. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB of the appropriate pH, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to FcRn by FACS™ using a FACSCalibur™ flow cytometer (BD® Biosciences). Mean channel fluorescence (MCF) of each mutant was plotted using Excel (Microsoft® Corporation).

Please replace the paragraph at page 74, lines 18-29, with the following amended paragraph:

The antigen binding activity of Hu1D10-IgG2M3 wild-type and mutant antibodies was confirmed in a FACS™ binding assay using Raji cells, which express an allele of the HLA-DR β chain that is recognized by Hu1D10 (Kostelny et al. (2001), op. cit.). Approximately  $2.5 \times 10^5$  cells/test were washed once in FBB, pH 7.4, and resuspended in 140 µl of Hu1D10-IgG2M3

antibody (threefold serial dilutions from 60 µg/ml to 0.027 µg/ml) in FBB, pH 7.4. The cells were incubated with antibody for 1 hour on ice, washed twice in FBB, pH 7.4, and resuspended in 25 µl of goat F(ab')<sub>2</sub> anti-human kappa RPE-conjugated antibody (Southern Biotechnology Associates, Inc.) diluted to 10 µg/ml in FBB, pH 7.4. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB, pH 7.4, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to the HLA-DR β chain allele by FACS™ using a FACSCalibur™ flow cytometer (BD® Biosciences).

Please replace the paragraph beginning at page 74, line 30, with the following amended paragraph:

Similarly, the antigen binding activity of Hu1D10-IgG1 wild-type and mutant antibodies was confirmed in a FACS™ binding assay using Raji cells. Approximately  $2.0 \times 10^5$  cells/test were washed once in FBB, pH 7.4, and resuspended in 100 µl of Hu1D10-IgG1 antibody (twofold serial dilutions from 25 µg/ml to 12.5 µg/ml, then threefold serial dilutions from 12.5 µg/ml to 0.0020 µg/ml) in FBB, pH 7.4. A dilution series of HuFd79-IgG1 antibody (Co et al., Proc. Natl. Acad. Sci. 88:2869-2873 (1991)) was prepared as described above and used as a negative control. The cells were incubated with antibody for 1 hour on ice, washed twice in FBB, pH 7.4, and resuspended in 25 µl of goat F(ab')<sub>2</sub> anti-human IgG FITC-conjugated antibody (Southern Biotechnology Associates, Inc.) diluted to 20 µg/ml in FBB, pH 7.4. After incubation for 30 minutes on ice in the dark, the cells were washed twice in FBB, pH 7.4, and resuspended in 1% formaldehyde. Samples were analyzed for antibody binding to the HLA-DR β chain allele by FACS™ using a FACSCalibur™ flow cytometer (BD® Biosciences).